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Carbapenemase producing *Enterobacteriaceae* in intensive care units in Ecuador: Results from a multicenter study

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ABSTRACT

Introduction: Carbapenemase-producing *Enterobacteriaceae* (CPE) are of global concern due to the growing number of patients who acquire them and their association with high mortality rates. Although there are some reports of endemicity in developing countries, little is known about this microorganism, and Ecuador is not an exception. Subsequently, our objective was to clinically and molecularly characterize carbapenemase producing-*Enterobacteriaceae* in intensive care units (ICUs) in Guayaquil, Ecuador.
Methods: To determine CPE colonization, we obtained perineal and inguinal swabs from patients admitted to seven intensive-care adult units in Guayaquil-Ecuador between February and April 2016. The Centers for Disease Control and Prevention (CDC) laboratory protocol and chromogenic agar were used to process the cultures. Polymerase chain reaction was used to confirm carbapenemase production. Genotypic analysis was performed by Multilocus Sequence Typing (MLST) and pulsed-field electrophoresis (PFEG). Demographic and clinical data were obtained from the electronic charts and patient's relatives.
Results: Six hundred seventy-seven patients were included in the study, of whom 255 were colonized/infected by CPE. The CPE prevalence was 37.67%. Previous use of antimicrobials, use of invasive procedures and being burned at admission were associated with CPE. The most frequent infection was found after a surgical procedure. *Klebsiella pneumoniae* (n = 249) was the predominant microorganism harbouring bla_{KPC}, followed by *Enterobactercloacae* (n = 8), *Klebsiella aerogenes* (n = 4), *Escherichia coli* (n = 4) and *Klebsiella oxytoca* (n = 1). NDM was present in *Proteus mirabilis*. The strains were distributed in 19 sequence types (ST), and 10 were not reported previously in Ecuador. ST 258 was the sequence type isolated most frequently.
Conclusion: This study shows a high prevalence of CPE in ICUs, particularly *K. pneumoniae* bla_{KPC} ST 258. The identification of KPC alleles may help to understand the routes of dissemination and control spread within ICUs in Guayaquil, Ecuador.

Background

Carbapenemase-producing *Enterobacteriaceae* (CPE) are microorganisms resistant to the last resort antimicrobials for managing critically ill patients, and they have emerged as a cause of global epidemics worldwide. Therefore, the World Health Organization (WHO) has included them in a priority list for developing novel antimicrobial treatments [1].

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CPE have emerged as a substantial cause of morbidity and mortality (50%) because of their limited therapeutic options [2,3], which highlights the importance of preventing their transmission in healthcare facilities [4,5]. A large variety of carbapenemases have been identified in this family, belonging to three classes of ß-lactamases: The most clinically significant are KPC, IMP, VIM, NDM, and OXA-48. Most of the carbapenemases were identified in *K. pneumoniae* isolates that caused nosocomial outbreaks [6].

The CPE prevalence varies according to region and the carbapenemase type studied. Greece, Italy, and Israel are considered endemic areas for carbapenemase producing-*Klebsiella pneumoniae* (KPC), with incidences exceeding 30% [7,8]. India has a prevalence of 50% for NDM [9]. In South America, Colombia, Argentina, and Brazil have reported endemic KPC. Turkey and other Mediterranean countries have reported the OXA-48 variant as the most predominant in the region [8,10].

The National Surveillance Antimicrobial Resistance Program in Ecuador reported imipenem and meropenem resistance of approximately 1.4 and 2% in *Escherichia coli* and 20% *in K. pneumoniae* in blood samples [11].

The vast majority of these isolates are characterized genetically as multilocus sequence type 258 (ST258) and are presumed to be clonally related by descent [12]. To our knowledge, there is scarce information about the behaviour of these microorganisms studied systematically in intensive care units (ICUs) in the country; subsequently, our objective was to clinically and molecularly characterize carbapenemase producing-*Enterobacteriaceae* in ICUs in Guayaquil, Ecuador.

Material and methods

Study population

This observational prospective study was carried out in seven intensive-care adult units in Guayaquil, a city with a population of 4.5 million of inhabitants, located in Ecuador. The study period was between February and April of 2016. For surveillance purposes, inguinal and perineal swabs were performed weekly for all patients in the ICUs. The first sample was obtained 48 h after entry and on admission for those who reported previous hospitalization.

Data collection

The demographic and clinical data were collected through electronic records and the patient's relatives. The measured variables were age, sex, ICU entry date, length of the previous hospital stay, comorbidities, diagnosis on admission, acute physiology and chronic health evaluation (APACHE II) score, and the diagnosis of healthcare-associated infections (HAIs). The HAI definition was adopted from the Centers for Disease Control and Prevention in Atlanta (CDC) [13]. Invasive infections were determined when an organism was isolated from a normally sterile body fluid or deep tissue [14].

Microbiological cultures

We processed samples by the CDC method, according to a previously described methodology [15], using CHROMagar mSuper CARBA™ agar (CHROMagar™, France).

All colonies suspected to harbour CPE were identified with the Api 20 E system™ (BioMérieux, France). An antimicrobial susceptibility test was performed with the disk diffusion method with 10 μg imipenem (IMP) and 10 μg meropenem (MER) (Liofilchem, Italy) following the Clinical Laboratory Standards Institute (CLSI) guidelines [16]. Isolates showing inhibition zones ≤ 22 mm were considered carbapenem-resistant [17].

We tested all carbapenem resistance *Enterobacteriaceae* (CRE) with a microbiological inactivation method (mMIC) [18] and combined-disk tests of carbapenems with/without phenylboronic acid (PBA) and EDTA (Liofilchem, Italy) [19]. Carbapenemase production was confirmed phenotypically if the mMIC and combined-disk test with PBA or EDTA were positive.

CRE with an mMIC and/or a combined-disk negative test were also tested with a modified Hodge test in Mueller Hinton agar (Becton Dickinson, UK), which was supplemented with cloxacillin sodium salt (250 mg/100 ml) (MHT-C) (Sigma Aldrich) and a meropenem disk (10 μg) [20]. We defined CRE non-producing carbapenemase (CRE non-PC) as the mCIM and MHT-C negatives isolates. These isolates were also studied for extended spectrum ß-lactamase (ESBL) and AmpC production. An ESBL phenotype was defined in *Enterobacteriaceae* with a synergistic effect observed between cefepime (FEP 30 μg), cefotaxime (CTX 30 μg) or ceftazidime (CAZ 30 μg) disks and an amoxicillin/clavulanic acid disk (CLAV 20 μg/10 μg), which was placed 15 mm from centre to centre of CAZ, FEP and CTX [19]. An AmpC phenotype was defined as CRE non-PC isolates with a synergistic effect observed with the double disk method with ertapenem (ERT 10 μg) and phenylboronic acid disks (300 μg) (PBA) (Kirby Bauer method with ERT and APB disks placed 15 mm from centre to centre in Mueller Hinton agar supplemented with 250 mg/ml cloxacillin sodium salt).

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603 and *K. pneumoniae* ATCC BAA-1705 were used as quality control strains.

Every hospital's laboratory processed their clinical samples according to conventional microbiological protocols, and the strains were studied phenotypically for carbapenemase production.

Control measures

All carbapenem resistant *Enterobacteriaceae*-positive patients were placed in contact precautions isolation according to the facility protocol. A 4% chlorhexidine soap was used for their daily hygiene, and no staff cohorting was performed. Healthcare personnel caring for patients on Contact Precautions wore a gown and gloves for all interactions that involved contact with the patient or potentially contaminated areas in the patient's environment. Staff working in the affected units received education seminars and attended workshops on hand hygiene and patient isolation. The units were cleaned thoroughly with two products that were also used for daily disinfection and contained potassium peroxymonosulfate.

Molecular carbapenemase identification

The presence of the *bla*KPC, *bla*OXA-48, *bla*VIM, *bla*IMP and *bla*NDM genes were molecularly confirmed in all CPE through a multiplex polymerase chain reaction (PCR) [21].

Clonal relatedness and diversity analysis

The genetic relatedness between *K. pneumoniae* bla_{KPC} was determined by ERIC-PCR according to the reaction conditions previously described [22]. No other carbapenemase was studied.

Next, a strain from each electrophoretic pattern obtained with ERIC-PCR was analysed with pulsed-field electrophoresis (PFGE) and the restriction enzyme XbaI (https://www.cdc.gov/pulsenet/pdf/ecoli-shigella-salmonella-pfge-protocol-508c.pdf). PFGE was performed with a (Bio-Rad) CHEF DR III System at 6 V/cm, 14 °C, 120 °C included angle, with an initial switching time of 6.76 s to a final switch time of 35.38 s, 5 to 15 s for 18 h. The clonal analysis was performed with BioNumerics software version 6.1 (Applied Biosys-

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tem), and the analysis of similarity was performed with the Dice coefficient. Related dendograms were generated with the UPGMA method (optimization parameter 1.5 and tolerance 2% for ERIC-PCR and 1.8% for PFGE). The strains with similarity > = 80% were assigned to the same group in ERIC-PCR and PFGE.

Determination of KPC variant

Purification of the *bla*KPC PCR products was performed following the manufacturer's instructions (BigDye (R) X terminator (TM) Purification kit Protocol, Applied Biosystem). Amplicons were sequenced by MACROGEN INC. Korea (http://www.macrogen.com/esp/service_sequ02.html). Sequence alignment was performed with MEGA 6 software and analysed with the "Basic Local Alignment Search Tool (BLAST)" database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). We included two isolates from ST 258, ST 512 and ST 45. The other ST isolates were not further analysed to determine the KPC variant.

Multilocus sequence typing

The sequence typing (ST) of the *K. pneumoniae* isolates was determined by Multilocus sequence typing (MLST) using a previously described protocol (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). The ST assignment was made with the *Klebsiella* MLST database (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html).

Statistical analysis

Data analysis was performed using Software R version 3.5.0. The association between the CRE patients and the categorical variables was first examined using a Chi-square test. When expected cell sizes are lower than 5, we circumvent the problem using a Fisher's exact test. For both tests we set a significant level of $\alpha = 0.05$. For the first model, we studied 95% confident intervals for the odd-ratios (ORs) while for the second model, we considered a p value lower than α (0.05) to be statistically significant.

Ethical approval

The study was approved by the Ethics Committee of the Catholic University of Santiago de Guayaquil. An informed consent form was signed by each patient or their relative.

Results

CPE prevalence and patient characteristic

The present study included 148 intensive care beds from 4 private and 3 public hospitals. Three of the hospitals did not have a microbiology laboratory, therefore their microbiological samples were processed in laboratories outside the health-care unit. Detailed information about participating hospitals is shown in Table 1.

In the study period, 677 patients were enrolled. Two hundred and fifty-five patients (37.67%, 255/677) had at least one carbapenemase producing-*Enterobacteriaceae*. Two hundred eight patients were colonized by CPE and forty-seven (18.43%) had at least one HAI. Thirty-one patients had an invasive infection and four patients had more than one infection. The distribution of the HAIs were: surgical site infections (n = 14,26.92%), ventilator associated pneumoniae (n = 14,26.92%), catheter-associated urinary tract infections (n = 6, 11.54%), primary bloodstream infections (n = 5, 9.62%), pressure ulcer infections (n = 4, 7.69%), burned wound infections (n = 4, 7.69%), skin and soft tissue infections (n = 3, 5.77%) and one case of healthcare associated pneumonia (1.92%). Thirty-three patients (70%) were colonized prior to being infected.

The median time from admission to CPE detection was 15.36 days (2–191 days). Table 2 shows the patient clinical characteristics. Chronic heart failure ($p = 0.01$) and burns ($p = 0.00$) were significantly associated with CPE. Other admission diagnostics did not show statistical significance. Burns and connective tissue diseases had higher OR values (4.31 and 2.58, respectively). Comorbidities were also not associated with the acquisition of CPE ($p > 0.05$), with the exception of connective tissue diseases ($p = 0.02$) and paraplegia, hemiplegia or quadriplegia ($p = 0.04$). The risk factors for acquiring CPE were mechanical ventilation (OR: 3.07, IC 95% 2.11–4.48, $p = 0.0$), central venous catheter (OR: 2.76, IC 95% 3.07, $p = 0.0$), urinary catheter (OR: 4.03, IC 95% 1.52–6.19, $p = 0.0$), gastrostomy (OR: 2.41, IC 95% 2.41, $p = 0.0$), tracheostomy (OR: 5.09, IC 95% 3.47–7.48, $p = 0.0$), nasogastric tube (OR:2.57, IC 95% 1.76–3.76, $p = 0.0$), haemodialysis catheter (OR: 2.01, IC 95% 1.2–3.4, $p = 0.0$), surgery (OR: 1.82, IC 95% 1.33–2.49, $p = 0.0$) and parenteral nutrition (OR 2.05, IC 95% 1.47–2.86, $p = 0.0$). Approximately 43.57% of the population received vancomycin, 37.07% carbapenems, 30.43% piperacillin/tazobactam, 15.21% cephalosporins, 13.45% fluoroquinolones, 2.66% amynoglucosides, 4.60% macrolides and 0.74% aztreonam. Carbapenems (OR:3.06, IC 95%2.2–4.24, $p = 0.0$), vancomycin (OR: 3.57, IC 95%2.58–4.94, $p = 0.0$) and macrolides (OR: 2.75, IC 95% 1.31–5.77, $p = 0.0$) were associated with CPE. Ampicillin-sulbactam (OR: 1.17, IC 95%0.83–1.65, $p = 0.37$), cephalosporin (OR:0.96, IC 95% 0.62–1.48, $p = 0.35$), fluoroquinolones (OR:0.88, IC 95% 0.56–1.4, $p = 0.59$), aminoglycoside (OR;0.47, IC 95% 0.15–0.17, $p = 0.17$), piperacillin-tazobactam (OR:2.75, IC 95% 1.24, $p = 0.2$) and aztreonam (OR:0.41, IC 95% 0.05–3.7, $p = 0.41$) were the antimicrobial agents that do not show any statistical significance.

Clinical conditions associated with colonization and infection are shown in Table 3. Tracheostomy (OR 0:4, IC 95% 0.21–0.76, p = 0.0), surgery (OR:0.16, IC 95% 0.06–0.38, p = 0.0) and parenteral nutrition (OR: 0.47, IC 95% 0.25–0.9, p = 0.02) were associated with HAIs.

Table 1
Prevalence of CPE and characteristics of participating hospitals.

Hospital code	Attention level	Type of attention	No hospital bed	Hospitalization days	No hospital discharges	No ICUs beds	Microbiology laboratory	No of CPE (%)
1	Third	Private	101	27.781	7.656	15	Yes	17 (6.7%)
2	Third	Public	117	17.313	2.068	4	Yes	5 (2.0%)
3	Third	Public	598	262.181	24.205	85	Yes	181 (71.0%)
4	Second	Private	18	7.583	2.464	8	No	6 (2.4%)
5	Third	Public	126	46.36	9.583	18	No	12 (4.7%)
6	Second	Private	34	7.627	2.018	9	No	24 (9.4%)
7	Third	Private	101	25.28	5.57	9	Yes	10 (3.9%)
Total			1095	394.125	53.564	148		255

CPE: carbapenemase producing-*Enterobacteriaceae*; ICU: intensive care unit; Bla: β-Lactamase: KPC: *K. pneumoniae* carbapenemase.

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Table 2
Patient characteristics in ICUs in Guayaquil-Ecuador.

Variables	CPE positive (n = 255)	CPE negative (n = 422)	OR (95% IC)	p value
Sex (M)	155 (60.78%)	241 (57.11%)		0.34701
Mean age (years) (±SD)	53.68 (±19.81)	55.11(±20.52)		0.18506
APACHE II (±SD)	16.16 (±8.42)	13.42 (±8.61)		0.00003
Transfer	117 (45.88%)	126 (29.86%)	1.99 (1.44–2.75)	0.00003
ICU stay (±SD)	25.15 (±25.08)	11.78 (±10.05)		0.00000
Length of hospital stay (±SD)	34.33 (±26.8)	20.45 (±23.01)		0.00000
Mortality	95 (37.25%)	106 (25.12%)	1.77 (1.27–2.48)	0.00081
Admission diagnostic				
Renal failure	32 (12.55%)	40 (9.48%)	1.37 (0.84–2.24)	0.20925
Malignancy	4 (1.57%)	13 (3.08%)	0.5 (0.16–1.55)	0.22310
Diabetes mellitus	11 (4.31%)	21 (4.98%)	0.86 (0.41–1.82)	0.69384
Chronic heart failure	31 (12.16%)	82 (19.43%)	0.57 (0.37–0.9)	0.01391
Neurological disease	61 (23.92%)	82 (19.43%)	1.3 (0.9–1.9)	0.16546
Chronic lung disease	3 (1.18%)	13 (3.08%)	0.37 (0.11–1.33)	0.11403
Connective tissue disease	3 (1.18%)	2 (0.47%)	2.5 (0.41–15.06)	0.30091
Burn	19 (7.45%)	8 (1.9%)	4.17 (1.8–9.66)	0.00034
Immunosuppression				
HIV	5 (1.96%)	14 (3.32%)	0.58 (0.21–1.64)	0.30035
Use of steroids (>20 mg prednisone/15 days)	6 (2.35%)	5 (1.18%)	2.01 (0.61–6.65)	0.24408
Solid Organ Transplant	1 (0.39%)	1 (0.24%)	1.66 (0.1–26.62)	0.71847

APACHE II: acute physiology and chronic health evaluation II score, CPE: carbapenemase producing *Enterobacteriaceae*, ICU: intensive care unit, OR: odds ratio.

Table 3
Clinical conditions associated with CPE infection in ICUs in Guayaquil-Ecuador.

Variables	Colonized (n = 255)	Infected (n = 422)	OR (95% IC)	*P* value
Sex (M)	123 (59.13%)	32 (68.09%)		0.25634
Mean age (years)	54.34 ± 19.98	50.74 ± 18.96		0.18506
APACHE II	16.05+-8.38	16.64 ± 8.68		0.00003
Transfer	90 (43.27%)	27 (57.45%)	0.56 (0.3–1.07)	0.07813
ICU stay (±SD)	24.35 ± 26.66	28.72 ± 16.08		0.00000
Length of hospital stay (±SD)	34.03 ± 28.3	35.64 ± 19		0.00000
Mortality	74 (35.58%)	21 (44.68%)	0.68 (0.36–1.3)	0.24366
Admission diagnostic				
Chronic heart failure	31 (12.16%)	82 (19.43%)	0.57 (0.37–0.9)	0.01391
Neurological disease	61 (23.92%)	82 (19.43%)	1.3 (0.9–1.9)	0.16546
Burn	19 (7.45%)	8 (1.9%)	4.17 (1.8–9.66)	0.00034

APACHE II: acute physiology and chronic health evaluation II score, CPE: carbapenemase producing *Enterobacteriaceae*, ICU: intensive care unit, OR: odds ratio.

CPE was found in all the participating hospitals. The presence of colonization/infection was significantly higher in public hospitals than in private ones (p < 0.000), as well as in tertiary hospitals (p < 0.000). The prevalence of CPE per unit is shown in Table 1.

Bacterial isolates and carbapenemase characterization

A total of 1146 inguinal and perineal swabs (range 1–6 per patient; median 2) were obtained. SuperCarba Chromoagar and the CDC method were both used for microbiological cultures of 950 specimens (605 patients). One hundred and sixty-six samples used the CDC method as the technique for culture processing due to economic limitations. Carbapenemase production was the most frequent mechanism of carbapenem resistance (n = 255), and CRE-non-producing carbapenemase (CP) were isolated in 27 patients.

The predominant carbapenemase type identified was KPC (91.72%), followed by NDM (2.65%). None OXA-48, IMP or VIM were found.

K. pneumoniae was the predominant microorganism (n = 249) harbouring bla$_{KPC}$, followed by *E. cloacae* (n = 8), *K. aerogenes* (formerly *E. aerogenes*) (n = 4), *E. coli* (n = 4) and *K. oxytoca* (n = 1). NDM was present only in *P. mirabilis*. Table 4 shows the molecular mechanism of carbapenem resistance and the carbapenemase genotype detected.

Fifty-seven electrophoretic patterns were distributed in twenty-two clusters from 174 isolates of *K. pneumoniae* bla$_{KPC}$ by ERIC-PCR (Fig. 1). PFGE identified seven clusters among 108 isolates analysed (including 57 patterns from ERIC-PCR and 51 isolates not included in ERIC-PCR). Two PFGE clusters were predominantly detected by using a cut-off of 80% similarity in the hospital units studied. Cluster A (n = 45; 17 subtypes) and cluster B (n = 40; 7 subtypes) included 41.67% and 37.03% of the isolates, respectively. The rest of the isolates were distributed in cluster C (n = 1; 2 subtypes), D (n = 15; 6 subtypes), F (n = 1), G (n = 2) and J (n = 4; 2 subtypes) (Fig. 2). Detailed data of the cluster subtypes are presented in Fig. 3.

Table 4
Resistance mechanism of CPE and CRE non-PC.

Species	CPE			CRE non-PC	
	Carbapenemase genotype				
	KPC	NDM	Total	ESBL + porin mutations	AmpC + porin mutations
K. pneumoniae	249	0	249	9	4
K. aerogenes	4	0	4	0	1
K. oxytoca	1	0	1	0	0
E. coli	4	0	4	2	0
E. cloacae	8	0	8	1	1
P. mirabilis	0	8	8	0	0

CPE: carbapenemase producing *Enterobacteriaceae*, CRE non-PC: carbapenem resistant *Enterobacteriaceae* non-producing carbapenemase, KPC: *Klebsiella pneumoniae* carbapenemase, NDM: New Delhi metallo-β-lactamase, ESBL: extended spectrum ß-lactamase.

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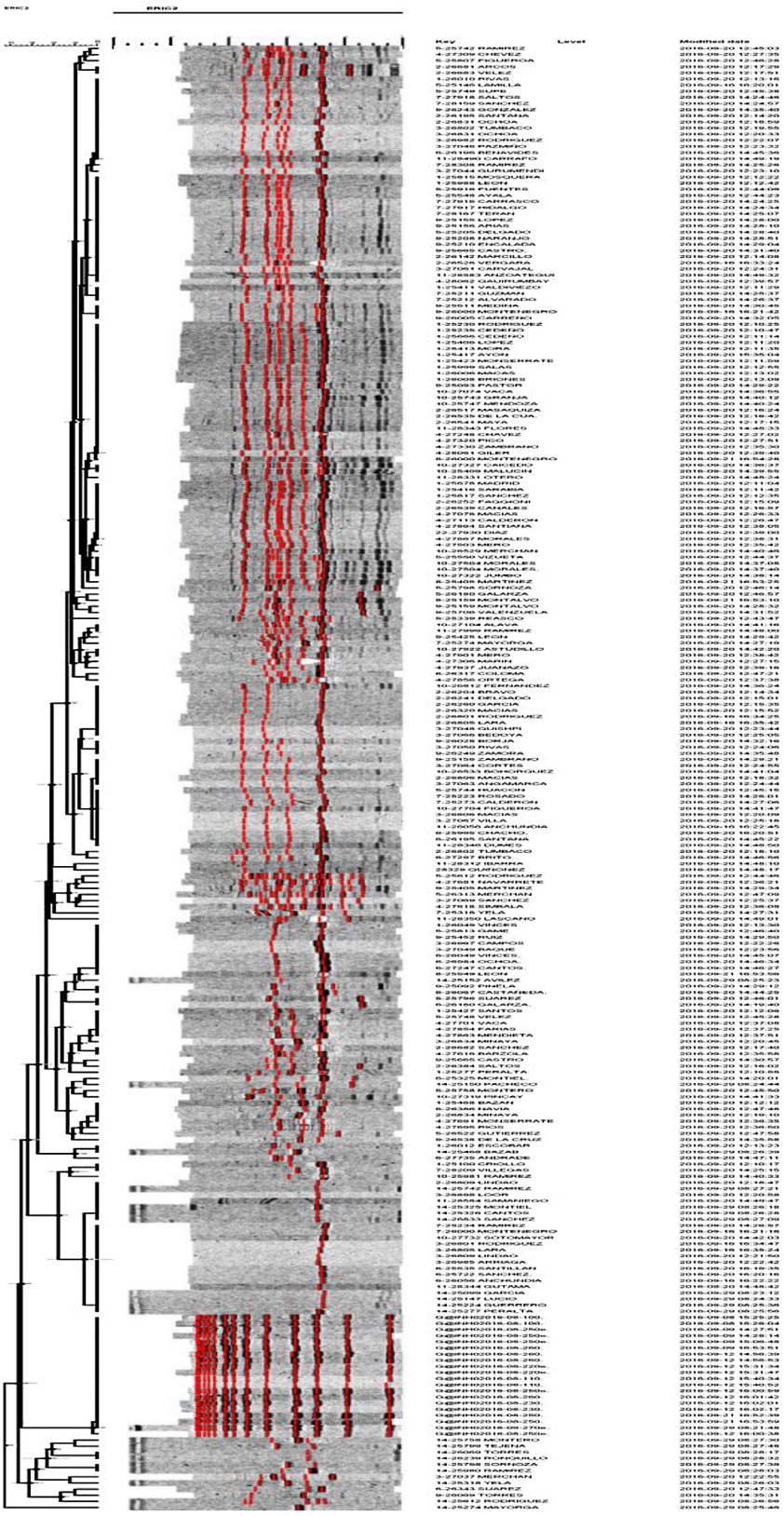

Fig. 1. Dendogram of ERIC_PCR results for all *K. pneumoniae* isolates. The scale at the top represents the genetic distance between isolates.

Twenty-two sequence types were obtained from 37 isolates studied. Sequence type 258 (n = 5) was the predominant ST found. Other STs were ST 512 (n = 3) and ST 45 (n = 3). Sequence type 25, 35, 39, and 307 had two isolates each. Eighteen isolates belonged to ST 1, 34, 36, 37, 42, 54, 70, 151, 231, 526, 628, 659 and ST 1040, each ST with one isolate. Four *K. pneumoniae* bla_{KPC} were not typed according to the MLST database, therefore more studies should be performed. bla_{KPC}-2 was present in ST-258, ST512 and ST 45. Fur-

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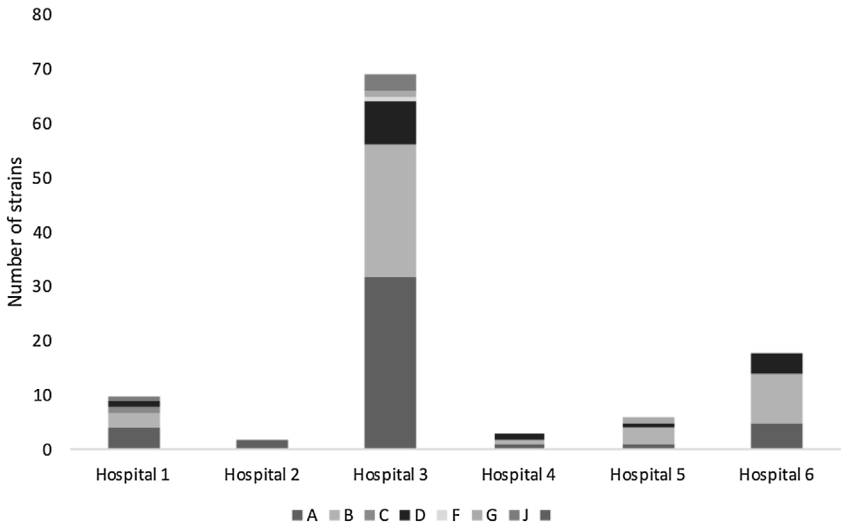

Fig. 2. Pulse-field electrophoresis patrons distribution.

ther studies in isolates harbouring bla_{NDM} and in the other sequence types were not performed because of economical limitations.

Discussion

CPE constitute a public health threat worldwide due to their high levels of antimicrobial resistance associated with high mortality rates [4]. In Ecuador, the first infection caused by *K. pneumoniae* with carbapenemase type KPC-2 was reported in 2013 [23], and since then, there has been rapid dissemination of the bacteria around the country [11]. During the study period, the CPE prevalence was high (37,7%). Furthermore, the presence of CPE was associated with higher morbidity and mortality rates. In our study, *K. pneumoniae* bla_{KPC} was the carbapenemase most frequently isolated (91.72%).

CPE was associated with prior exposure to carbapenems, vancomycin, and macrolides, as well as the presence of invasive devices and surgery. At admission, burn patients had a significant risk for acquiring CPE.

The CPE prevalence in our study was much higher than those published in developed countries [8,24] and Latin American countries, such as Argentina or Brazil, accounting for a 25% prevalence [10,25]. It is important to note that our work focused on ICUs, where the prevalence is expected to be higher than in other units because of the complexity and higher APACHE II scores of the patients admitted.

This study showed higher mortality rates in CPE colonized/infected patients than in CPE negative patients but did not demonstrate differences between CPE colonized and infected patients. These results differ from those reported by other authors, where the length of the ICU stay was considered an independent risk factor, but not KPC colonization [26,27]. Colonization is a well-known risk factor for a worse outcome; a study performed in Greek intensive care units showed that CPE colonized patients are at a 1.79 times more significant risk of dying than non-colonized patients [28]. Active carriers monitoring has been successfully implemented in multifactorial strategies aiming to limit the spread of CPE [29], and it should be considered as part of a coordinated regional effort to reduce endemic CPE.

Previous studies have demonstrated an association between KPC-producing *K. pneumoniae* infection and the length of hospitalization, use of central venous catheters, ICU stay, and exposure to specific broad-spectrum antimicrobial agents, such as carbapenems, cephalosporins, fluoroquinolones, and penicillin, as well as having surgery [30]. Macrolides and vancomycin have not been considered risk factors in other studies, and to our knowledge, this study is the first that shows an association between burns and CPE. These patients usually have long term stays and sometimes required multiple procedures including surgeries, which could have increased the risk of infection by KPC. Surgical site infections, considered the most important healthcare-associated infections, had the highest occurrence, suggesting that hospitals should emphasize hand hygiene strategies, sterilization of surgical instruments and the disinfection of operating rooms.

The prevalence of CPE was higher among health-care units in tertiary care clinics than in secondary care clinics, which could be explained by the severity and complexity of the patients admitted in tertiary clinics. There is little information about this topic. Most of the data are related to studies performed in single hospitals rather than in different types of institutions [28–31]. In three of the hospitals included in the study, CPE diagnostics were performed through external laboratories. No hospitals were performing weekly surveillance cultures at the time the study was carried out, and both situations could contribute to the high prevalence rates and result in limited notification of outbreaks.

Several nosocomial outbreaks, most often due to *K. pneumoniae*, have been reported in the United States, Colombia, Argentina, Greece, Italy, Poland, China, and Israel [32]. KPC producing bacteria are considered to be endemic in certain parts of the world, such as Colombia, and are important causes of nosocomial acquired infections [33]. In Ecuador, KPC-2 is the carbapenemase that has been reported most frequently [24,34]. Considering our results, KPC-2 could be endemic in our city. It is important to highlight that OXA had not been detected in our hospitals until this study, in contrast to other countries that reported this carbapenemase as the most frequent [32]. PFGE analysis showed a clonal circulation of carbapenemase-producing *K. pneumoniae*, with an intra- and inter-hospital spread in the intensive care units in Guayaquil city. ST 258 was the most frequent sequence type recognized, and these results are similar to those presented by Benavides et al. in the samples studied from the National Institute of Research in Ecuador and from other regions worldwide [35]. ST 25 has been described previously by Tamayo et al. in our country [36]. It is important to note that ST 512, 45, 35, 307, 1, 34, 36, 37, 42, 54, 70, 151, 231, 526, 628, 695, and 1040 are reported for the first time in Ecuador.

The short follow-up period, as well as the inability to confirm carbapenemase genotype in clinical samples, are the major limitations of this study. Chromogenic agar was used in the majority of

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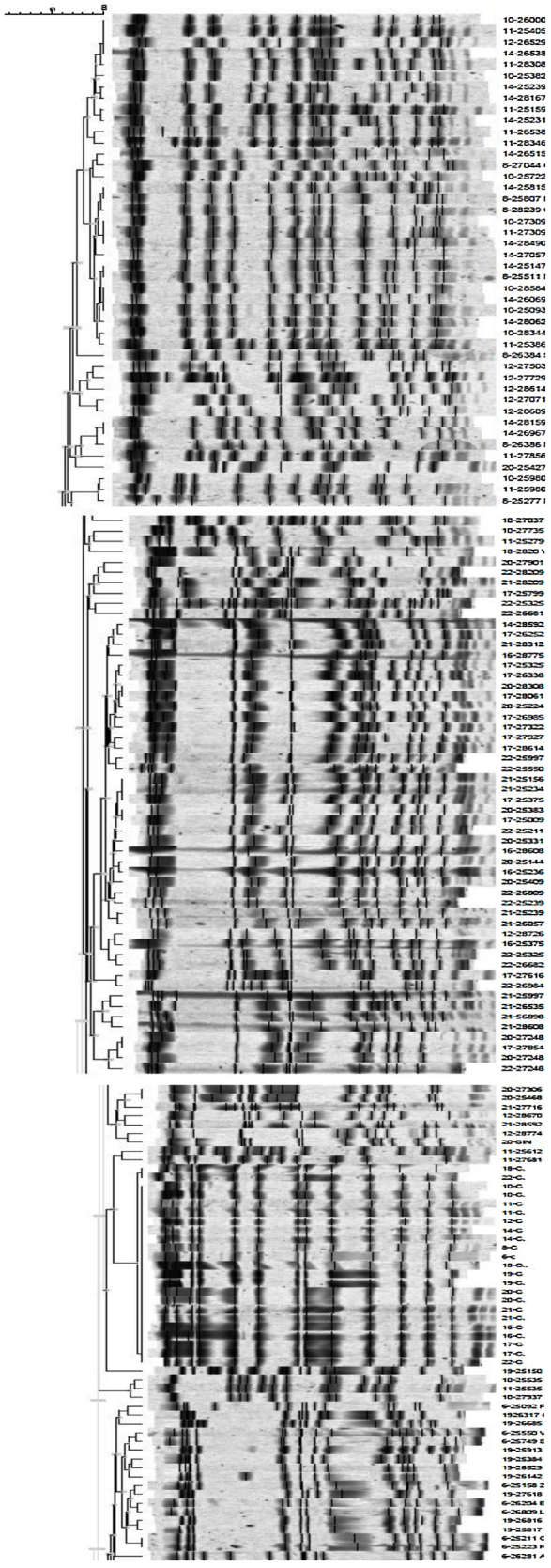

Fig. 3. Dendogram of the PFEG results for all *K. pneumoniae* isolates. The scale at the top represents the genetic distance between isolates.

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the samples, but not all of them, so results for carbapenemases such as OXA-48 or NDM could be misleading due to the low sensitivity of the CDC method [37].

Conclusion

This study shows a high prevalence of CPE in ICUs, particularly *K. pneumoniae* $bla_{KPC,-2}$ ST 258. The identification of KPC alleles in the other STs may help explain the routes of dissemination and how to control spread within hospitals in Guayaquil, Ecuador.

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Conflict of interest

S. C. is speaker for Merck and 3M. The other authors declare no conflict of interest.

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